

ROLAND CARLSSON

ALPHA-FETOPROTEIN:
ASPECTS ON FUNCTION AND SYNTHESIS

LUND 1981

From the Department of Zoophysiology, University of Lund,
Lund, Sweden

ALPHA-FETOPROTEIN: ASPECTS ON FUNCTION AND SYNTHESIS

by

ROLAND CARLSSON

LUND 1981



To Anne-Christine
and Anders



Digitized by the Internet Archive
in 2026

This dissertation is based on the following papers and on some additional observations:

- I. Carlsson, R.N.K., Ingvarsson, B.I. and Karlsson, B.W. (1976) Isolation and characterization of alpha-fetoprotein from foetal pigs. *Int. J. Biochem.* 7, 13-20.
- II. Carlsson, R.N.K., Ingvarsson, B.I. and Karlsson, B.W. (1977) Isolation and characterization of albumin from porcine serum, colostrum and urine. *Int. J. Biochem.* 8, 285-294.
- III. Ingvarsson, B.I. and Carlsson, R.N.K. (1978) Binding of fatty acids and tryptophan to alpha-fetoprotein from fetal pigs. *Biochim. Biophys. Acta* 537, 507-509.
- IV. Carlsson, R.N.K., Estes, T., DeGroot, J., Holden, J.T. and Ruoslahti, E. (1980) High affinity of alpha-fetoprotein for arachidonate and other fatty acids. *Biochem. J.* 190, 301-305.
- V. Carlsson, R.N.K. and Ingvarsson, B.I. (1979) Localization of alpha-fetoprotein and albumin in pig liver during fetal and neonatal development. *J. Dev. Biol.* 73, 1-10.
- VI. Carlsson, R., Engvall, E., Freeman, A. and Ruoslahti, E. (1981) Laminin and fibronectin in cell adhesion: Enhanced adhesion of cells from regenerating liver to laminin. *Proc. Natl. Acad. Sci. USA* 78, 2403-2406.

These papers will be referred to by their Roman numerals.

CONTENTS

INTRODUCTION	7
HISTORY OF ALPHA-FETOPROTEIN	7
PURIFICATION OF AFP	8
PHYSICOCHEMICAL PROPERTIES OF AFP	9
Developmental and clinical aspects on lectin-	
reactivity of AFP	10
Lectinreactivity of porcine AFP	11
CHEMICAL SIMILARITIES BETWEEN AFP AND ALBUMIN	14
BIOLOGICAL FUNCTION OF AFP	14
Immunosuppressive action of AFP	15
Functional similarities between AFP and albumin	17
MONOCLONAL ANTIBODIES TO AFP	20
SYNTHESIS OF AFP	22
During normal development	22
In cancer and nonmalignant liver injury	23
CONTROL OF AFP SYNTHESIS	25
SUMMARY AND CONCLUDING REMARKS	31
ACKNOWLEDGEMENTS	33
REFERENCES	34

Abbreviations used:

AFP	alpha-fetoprotein
Con A	concanavalin A
LCA	lens cullinaris agglutinin
SDS	sodium dodecyl sulfate
PHA	phytohemagglutinin
PWM	pokeweed mitogen
DEN	diethylnitrosamine

INTRODUCTION

During ontogenetic development an array of antigens are expressed transiently at specific developmental stages (Raftell and Perlmann, 1969 a, b). Large efforts to clarify the chemical nature, biological function and mode of synthesis of some of these embryonic antigens have been made over the past 20 years.

The aim of my study has been to shed some light on one of these embryonal or fetal antigens i.e. alpha-fetoprotein as concerns physicochemical and functional properties as well as mechanisms governing its synthesis.

HISTORY OF ALPHA-FETOPROTEIN

In 1956 Bergstrand and Czar described an α -globulin that was present in human fetal serum but absent from adult human serum. The protein was later named alpha-fetoprotein (AFP) and homologous proteins were subsequently found in all mammalian species studied in this respect including mice, rat (Watabe, 1974), rabbit (Branch, 1972), cow (Kithier and Poulik, 1972), sheep (Lai et al., 1977), dog (Nishi and Hirai, 1972), pig (Karlsson, 1970). AFP or AFP like proteins have also been found in nonmammalian species as in amphibia, reptiles, birds and fish (Gitlin, 1974).

The interest in AFP increased considerably after the discovery by Abelev et al. (1963) and Tatarinov (1964) that alpha-fetoprotein reappeared in serum of individuals suffering from primary liver carcinoma. Reexpression of gene products normally only expressed during fetal development has by now been recognized as a common phenomenon in tumors (for review see: Carcino-Embryonic proteins, Lehmann, ed., 1979). Such gene products have been termed carcino-embryonic, carcino-fetal or oncodevelopmental antigens. The existence of such antigens suggests similarities in the state of differentiation between fetal and tumor tissue and supports the concept that ontogenic

development is a process of variable expression of a stable genome (Davidson, 1976).

The fact that AFP is a fetal antigen, normally expressed only during a certain developmental stage, makes it a good candidate for studies on gene regulation. However, to understand the biology of an antigen it is first necessary to thoroughly investigate and understand the chemistry of that particular antigen. This can be exemplified by the fact that AFP often was mistaken for fetuin. Most of the confusion was shattered when Kithier and Poulik (1972) described that the two proteins were different gene products.

PURIFICATION OF AFP

The introduction of affinity chromatography greatly improved the purification of proteins from complex mixtures of protein like biological fluids. Molecules with specific affinity (ligands) were covalently coupled to matrices (Porath et al., 1967). For isolation of AFP groupspecific substances like concanavalin A (Con A) have been used (Pagé, 1973; Smith and Kelleher, 1973). Although chromatography on Con A will not produce pure AFP (paper I) it can often be of use as an initial step in purification of glucoproteins.

Preparations of AFP with higher purity are obtained when the specific interaction between antibody and corresponding antigen is utilized as in immunosorbent chromatography. The protein to be isolated is allowed to bind to its corresponding antibody on the insoluble matrix and is subsequently eluted (Nishi and Hirai, 1972; Pihko et al., 1973; Ruoslahti et al., 1974 a; paper I-IV). The other possibility is to absorb all contaminating proteins to the matrix and to allow the desired protein to pass through without coupling (paper I, II). This method has the advantage of never subjecting the protein to be isolated to extreme conditions, which often must be utilized for the elution of proteins bound to the

matrices. The method has the disadvantage of a low efficiency and can preferably be used as a last purification step for removal of minute contaminants (paper I-IV). A further improvement of the immunosorbent technique was recently described by Ruoslahti (1978). Recognizing the fact that the affinity of the immobilized antibody for the antigen should be low (Nishi et al., 1976; Ruoslahti, 1976) a preparation of antibodies with very low affinity was made. Antibodies to human AFP were absorbed with bovine AFP. The remaining antibodies with a very low affinity for bovine AFP were coupled to a solid phase of sepharose 4B and used for the isolation of bovine AFP under mild conditions. This technique was used for the isolation of the AFP in paper IV.

PHYSICOCHEMICAL PROPERTIES OF AFP

Due to the successful purification of AFP a thorough physico-chemical characterization of the molecule from many species has been possible. AFP is a single polypeptide with interchain disulfide bonds (Alpert et al., 1972; Pihko and Ruoslahti, 1973; Watabe, 1974). It has a molecular weight of about 70,000 daltons, a sedimentation coefficient of 4.5 - 4.7 and an isoelectric point ranging from 4.6 - 5.2 (Aliau et al., 1978; Alpert et al., 1972; Nishi, 1970; Ruoslahti et al., 1974 a; paper I). It is a glucoprotein as evidenced by its Con-A binding property (paper I) and it contains approximately 4 - 7 % carbohydrate (Aliau et al., 1978; Nishi, 1970; Ruoslahti and Seppälä, 1971; Watabe, 1974).

AFP-s from different species show immunological crossreactivity (Gitlin and Boesman, 1967; Nishi and Hirai, 1972; Nishi et al., 1975; Pihko et al., 1973; Zizkovsky and Masopust, 1974) and their amino acid composition is quite similar (Lai et al., 1977; Nishi, 1970; Pihko et al., 1973; Watabe, 1974; paper I). AFP exhibits a considerable microheterogeneity. This can be visualized on poly-acrylamide gel electrophoresis with and without sodium dodecylsulfate for rat and mouse AFP (Kerckaert et al., 1979; Ruoslahti and Engvall, 1978;

Watabe, 1974). No such electrophoretic microheterogeneity has been found for other AFPs including pig AFP (paper I). When analyzed by analytical isoelectric focusing the microheterogeneity becomes pronounced. Some but not all depends on variations in sialic acid content (Alpert and Perenchovich, 1975; Alpert et al., 1972) or in fatty acid content (Parmelee, 1978; paper IV). AFP shows microheterogeneity in its carbohydrate content. This manifests itself in differences in affinity for lectins, between subfractions of AFP (Bayard and Kerckaert, 1977; Smith and Kelleher, 1973). The microheterogeneity can be visualized on affinity chromatography of AFP on sepharose 4B columns coupled with lectins e.g. Con A. Typically Con A-bound, loosely bound and unbound fractions are obtained (Bayard and Kerckaert, 1977; Ruoslahti et al., 1978). A method originally developed by Bøgg-Hansen et al., (1975), crossed immuno-affinoelectrophoresis, has also been employed to resolve microheterogeneity in lectin reactivity.

Developmental and clinical aspects on lectin reactivity of AFP

AFPs from different animal species have their own characteristic pattern of lectin reactivity (Kerckaert et al., 1979). Developmental changes in the proportion of lectin e.g. Con A reactive and non-reactive fractions have been observed in AFP from man and mouse (Ruoslahti and Adamson, 1978; Ruoslahti et al., 1978). These changes are attributed to differences in glycosylation capacity between the two main AFP producing organs, the yolk sac which predominantly makes a Con A non-reactive AFP and the liver which makes a Con A reactive AFP (Ruoslahti and Adamson, 1978; Ruoslahti et al., 1978).

AFP obtained from amniotic fluid from early fetal development shows a high proportion of the Con A non-reactive form in man (Ruoslahti et al., 1978; Toftager-Larsen et al., 1980) whereas AFP from late fetal or neonatal development almost completely lacks Con-A non-reactive AFP. AFP from teratocarcinoma which embryologically

is a yolk sac tumor is high in the Con A non-reactive fraction whereas the hepatoma derived AFP is low in Con A non-reactive fraction (Ruoslahti et al., 1978).

It seems, however, that hepatomas produce AFP with a larger proportion of the Con A non-reactive variant than AFP produced by the normal fetal liver and by the adult liver in association with nonmalignant liver disease (Ruoslahti and Adamson, 1978; Ruoslahti et al., 1978). Amniotic fluid AFP from fetuses with malformations, e.g. spina bifida, shows a higher proportion of the Con A reactive variant than normal AFP. This is due to seepage of serum AFP which is predominantly Con A reactive. The differences in Con A reactivity between liver and yolk sac derived AFP can be utilized clinically for the diagnosis of fetal malformations that lead to increased communication between the fetal circulation and the amniotic fluid (Nørgaard-Pedersen et al., 1980; Smith et al., 1979; Toftager-Larsen et al., 1980). The interesting possibility that primary liver cancer could be diagnosed by its higher degree of Con A non-reactive AFP than AFP from nonmalignant liver disease needs further investigation.

Lectin reactivity of porcine AFP

The reactivity of swine AFP to lens cullinaris agglutinin (LCA) is similar to its Con A reactivity. However, generally a larger portion of the swine AFP is LCA non-reactive than Con A non-reactive (Fig. 1). The LCA non-reactive form in amniotic fluid is more prevalent during early fetal development than during the later stages of gestation (Fig. 2). The LCA reactivity of serum AFP from fetal pigs does not show any developmental pattern (Fig. 2). The same is true for the Con A reactivity. The proportion Con A non-reactive fraction of total AFP from fetal pig serum is essentially unchanged during at least the later half of the gestational period. The proportion non-reactive AFP is quite small, approximately 5-10% (not shown).

AFP in extracts of livers and whole fetuses from early fetal stages contains a high degree (50-55%) of the LCA non-reactive form (Fig. 3).



Fig. 1. Crossed immunoaffinity electrophoresis (CIAE) of porcine AFP. This and the following pictures were obtained in collaboration with Dr. Jean-Pierre Kerckaert INSERM, Lille, utilizing methods described earlier (Kerckaert et al., 1979). The first dimension of the plate contains the lectin, Concanavalin A (Con A) or Lens Culinaris agglutinin (LCA). The second dimension contains monospecific anti-porcine AFP (paper I). Reactivity of porcine AFP obtained from a one day old piglet to Con A (a) and to LCA (b).

These extracts are not contaminated by amniotic fluid and ought to be representative of the AFP that is present in serum. It should be

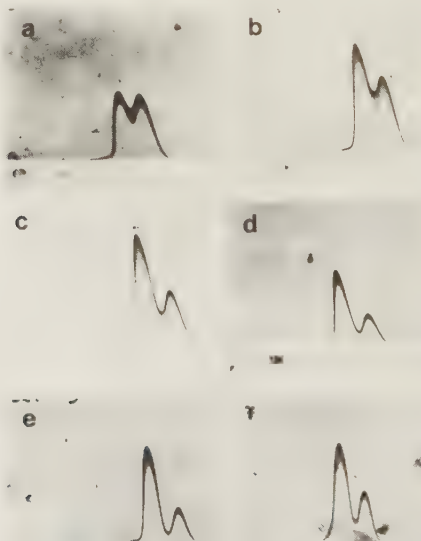


Fig. 2. LCA reactivity of porcine AFP from amniotic fluid; a-d and serum e-f from pig fetuses with various crown to rump lengths. a) 4 cm, b and e) 7 cm, c) 11 cm, d and f) 20 cm.

noted that occasionally single individuals within one litter have at late gestational stages serum-AFP that is up to 40% LCA non-reactive. The portion of LCA non-reactive AFP can even



Fig. 3. LCA reactivity of porcine AFP from extracts of whole fetuses. In (a) the fetuses measured 2,0 cm in (b) 2,5 cm from crown to rump.

be lesser in amniotic fluid than in serum from the same individual. This makes it difficult to discern any link between LCA-reactivity and yolk sac production of AFP.

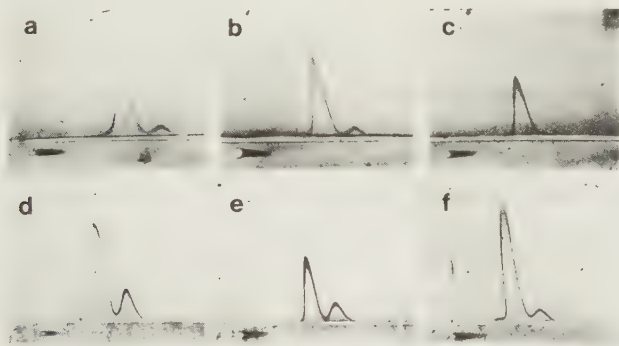


Fig. 4. LCA (a-c) and Con-A (d-f) reactivity of AFP from; (a) and (d) newborn unsuckled, (b) and (e) one day old and (c) and (f) four day old piglets.

Interestingly a successive decline in the proportion of both the Con A and LCA non-reactive forms are observed in serum derived swine AFP during neonatal development (Fig. 4). This could depend on a change in the relative rate of synthesis of the variants or on a faster catabolism of the non-reactive form. However, Barus

et al. (1978) showed for rat AFP that the Con A reactive and non-reactive variants are catabolized with the same rate. It can be calculated from the serum levels of AFP that there is a net increase in the amount of the non-reactive and of course in the reactive variant during early neonatal development. Although no conclusions concerning the reasons for the progressive decrease in the LCA or Con A non-reactive variants after birth can be drawn from this study it is not likely to be caused by a loss of an extra embryonic source of AFP but rather a progressive change in the way the liver glycosylates proteins. This might reflect a maturational process of the porcine liver. It should be noted that no such variation in glycosylation of rat AFP occurs during neonatal development of the rat (Smith et al., 1977).

CHEMICAL SIMILARITIES BETWEEN AFP AND ALBUMIN

The chemical relatedness between AFP and albumin has since long been indicated by their similar physicochemical properties (Grigorova et al., 1977; paper I, II). This view gained substantial support when it was found that antibodies made against denatured, unfolded AFP could cross-react with albumin (Ruoslahti and Engvall, 1976). In addition, Ruoslahti and Terry (1976) found that the two proteins showed homology in amino acid sequence. Their findings have been confirmed and extended by others (Innis and Miller, 1980; Liao et al., 1980 a). The primary structure of AFP has recently been established by deduction from the complete cDNA sequence (Innis and Miller, 1980; Law and Dugaiczky, 1981). A pronounced similarity in gene organization i.e. number and position of the intervening sequences between the AFP and albumin genes was demonstrated by Gorin and Tilghmann (1980). These findings strongly indicate that AFP and albumin are closely related from an evolutionary point of view and that they have evolved from a common ancestral gene (Jagodzinski et al., 1981).

BIOLOGICAL FUNCTION OF AFP

From the developmental pattern of serum AFP levels i.e. high concentra-

tions during fetal development, 3-10 mg per milliliter, and very low levels during adult life, 1-200 ng per milliliter, (Ruoslahti et al., 1974 a) it was proposed that AFP would play an important biological role in the fetal circulation.

That AFP has important functions was further suggested from studies in which injections of anti-AFP to pregnant rabbits have lead to abortion and death of their fetuses (Mizejewski and Grimley, 1976; Slade, 1973; Smith, 1972). Similarly Weller (1976) injected chicken embryos with anti-chicken AFP. Over 90 per cent of the embryos died within 2 hrs. However, these investigations have not distinguished between effects caused by reduced AFP-levels and cytotoxic effects caused by antibodies or antigen-antibody complexes. The results have been repudiated by others (Leung et al., 1977) who found no effect of antibodies to AFP on the survival of rat fetuses.

Immunosuppressive action of AFP

One of the earliest proposed functions of AFP was the immunosuppressive one (Murgita and Tomasi, 1975 a, b; Parmley and Hsu, 1973). The fetus is an allograft in relation to the mother but it is not rejected immunologically. A large number of investigators have demonstrated that AFP has immunosuppressive effects on T-cell dependent immune reactions in vitro (Murgita and Wigzell, 1976; Murgita et al., 1978 a; Lester et al., 1978 a; Yachnin and Lester, 1976). The inhibitory action has been reported to depend on bound estrogen (Keller et al., 1976) or on the content of sialic acid in the AFP (Zimmerman et al., 1977). However, Lester et al. (1978 b) found sialic acid to be without influence on the inhibitory action of AFP. In addition, human AFP which does not bind estrogen is suppressive in in vitro immune reactions thereby showing that bound estrogen is not a prerequisite for the suppressive action.

It has been suggested that AFP is immunosuppressive through a stimulating effect on T-suppressor cells (Murgita et al., 1977; 1978 b). However, whether AFP has an immunosuppressive effect or not is under debate as many

investigators have failed to demonstrate any such function (Hassoux et al., 1978; Littman et al., 1977; Sell et al., 1977; Sheppard et al., 1977; Soubiran et al., 1979). According to Pech et al. (1978) AFP is not generally immunosuppressive even on T-cell dependent immune reactions. Its suppressive action in mixed lymphocyte reactions is genetically restricted to antigens encoded by the I region. This might explain some if not all discrepancies between the findings of different investigators. Recently Ren and Chan (1981) reported a lack of correlation between the concentration of AFP in hepatoma serum and inhibition of immune response. They suggested that the immunosuppressive effect found with purified AFP was due to modifications of the AFP molecule during the process of purification and not relevant for the in vivo situation. Such modifications could easily occur in the amount and quality of bound substances e.g. fatty acids (see below).

When lymphocytes are stimulated by lectin mitogens like phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) they release arachidonic acid from phospholipids into the medium (Parker et al., 1979). These authors suggested that the generation of free arachidonic acid or metabolites of it would be involved in the activation process of the lymphocytes. It is possible that AFP through its high affinity for arachidonic acid could interfere in this mechanism by perhaps preventing reutilisation of released arachidonic acid and thereby suppress the mitogenic effect. However, even theoretically this explanation is not completely satisfactory. Parker et al. (1979) showed that a variety of mitogens including PWM caused release of arachidonic acid. Ren and Chan (1981) on the other hand showed that proliferation of lymphocytes induced by PWM could not be inhibited by purified AFP. Thus either the mitogenic response induced by PWM does not depend on metabolism of arachidonic acid or there is no link between release of arachidonic acid and proliferation of lymphocytes after mitogenic stimulation. Further investigations are needed to clarify the interrelationship between the ligand binding capacity of AFP and its ability to inhibit in vitro immune responses.

Functional similarities between AFP and albumin

The extensive molecular similarities between AFP and albumin, reviewed above, together with the inverse developmental pattern in serum-concentration of these two proteins during ontogeny (Abelev, 1971; Gitlin, 1975; Ingvarsson et al., 1978; Karlsson, 1972; Kekomäki et al., 1971; Ruoslahti and Seppälä, 1972; Sell et al., 1974; Stone, 1981) are consistent with the view that AFP might carry out some of the functions of albumin in the fetus.

Accumulated data suggest that AFP has properties similar to albumin as regards binding and transport of ligands. The first observation that AFP can bind small ligands was made by Endo et al. (1974) who showed that AFP bound dye substances. The binding property of AFP has subsequently been verified by a number of investigators showing that AFP can bind a variety of more naturally occurring substances e.g. metalions, bilirubin, estrogen and fatty acids. The similarity between AFP and albumin has thus been extended from mere physicochemical to functional properties.

Whether these in vitro findings have any biological significance in vivo has not yet been determined.

Binding of estrogen to AFP was demonstrated by Uriel et al., 1972. It was later demonstrated that only AFP from mouse and rat showed a specific high affinity for estrogen (Aussel and Masseyeff, 1978; Aussel et al., 1973; Soloff et al., 1976). AFP from other species including man, cow, rabbit, guinea pig, hamster and pig (Savu et al., 1974; Attardi and Ruoslahti, 1977; paper III) do not bind estrogen.

The biological significance of the estrogen-binding is not known. It has been suggested that binding of estrogen to AFP could protect the fetus from the mothers high estrogen levels (Savu et al., 1977) or influence growth and differentiation of brain cells (Benno and Williams, 1978; Sell et al., 1975; Sonnenschein and Soto, 1979; Soto and Sonnenschein, 1980).

In contrast to estrogen-binding the fatty acid binding property of AFP is shared by a number of species; rat (Benassayag et al., 1979, 1980), man (Berde et al., 1979; Parmelee et al., 1978), cow (Hsia et al., 1980; paper IV) and pig (paper III). That the binding of fatty acids is of some biological significance and not only an in vitro property has been validated by findings that AFPs like albumins isolated from serum of fetuses contain bound fatty acids (Benassayag et al., 1981; Parmelee et al., 1978; paper IV).

AFP and albumin do not bind fatty acids in an identical manner. Albumin has three binding sites with high affinity per molecule whereas AFP has only two (Versée et al., 1981; paper IV). AFP and albumin isolated from the same source exhibit differences in fatty acid content. AFP contains more long-chain polyunsaturated fatty acids than albumin (Benassayag et al., 1979, 1980; Parmelee et al., 1978; paper IV). AFP from rat and cow shows a higher affinity for long-chain polyunsaturated fatty acids such as arachidonic acid and docosahexaenoic acid (Benassayag et al., 1980; paper IV) than albumin. Observations suggesting that porcine AFP shares these binding characteristics, i.e. two binding sites per molecule with high affinity for long-chain polyunsaturated fatty acids are presented in Table I. However, Berde et al. (1980) using a competitive assay found that human AFP binds three moles of fatty acid per mole of protein and despite the fact that isolated AFP predominantly contains arachidonic acid and docosahexaenoic acid it does not show a higher affinity for these fatty acids. They also reported that human AFP bound two moles bilirubin per mole of protein whereas Ruoslahti et al. (1979), Hsia et al. (1980) and Versée and Barel (1979) only found one mole bilirubin per mole AFP.

It is not yet known if bilirubin, fatty acids and in rodents estrogen have common or separate binding sites on the AFP molecule. The data hitherto available on this aspect are somewhat confusing. It seems that the binding sites for bilirubin and estrogen on rat AFP are separate for and independent of each other (Versée and Barel, 1979). On the other hand Hsia et al. (1980) found that bilirubin and fatty acids share a binding site on bovine AFP. Versée et al. (1981) found that

Table I. Competition by porcine alpha-fetoprotein and albumin for the binding of fatty acids.

Initial concentration of fatty acid in the n-heptane phase									
Fatty acid	$3 \times 10^{-4} M$			$3 \times 10^{-5} M$			$1 \times 10^{-6} M$		
	$\bar{valb}$	$\bar{vAFP}$	$\frac{\bar{vAFP}}{\bar{valb}}$	$\bar{valb}$	$\bar{vAFP}$	$\frac{\bar{vAFP}}{\bar{valb}}$	$\bar{valb}$	$\bar{vAFP}$	$\frac{\bar{vAFP}}{\bar{valb}}$
16:0	5.26	2.05	0.40	2.17	1.25	0.58	0.09	0.11	1.22
18:1	4.40	1.77	0.40	1.46	1.06	0.73	0.11	0.14	1.27
20:4	5.10	2.70	0.53	1.90	1.78	0.94	0.06	0.23	3.83

Molar binding ratios for binding of palmitic (16:0), Oleic (18:1) and arachidonic acid (20:4) to defatted porcine alpha-fetoprotein $\bar{vAFP}$, and albumin $\bar{valb}$. Each value is the mean of four determinations that did not differ by more than 10%. AFP was isolated from fetal porcine serum utilizing crossreacting antibodies to bovine AFP immobilized on sepharose 4B principally as described by Ruoslahti (1978). The preparations of AFP were pure according to the criteria of paper I. Albumin was isolated from adult and fetal swine serum by preparative agarose gel electrophoresis. The partially purified albumin was then subjected to an anti-swine serum sepharose to remove contaminating proteins (paper II). The purity of the preparations was ascertained as described in paper II. The experiment was performed as described in paper IV. No differences in fatty acid-binding properties were found between albumins obtained from fetal or adult swine serum.

fatty acids could displace bound estradiol from rat AFP whereas estradiol could not displace bound fatty acids. It is possible that the binding sites for the various ligands partially overlap and/or that binding of one ligand will influence the binding of the other through conformational changes in the AFP molecule.

Further investigation is required to clarify whether the reason for the difference in the affinity of AFP from rat, cow and probably pig on the one hand and man on the other is a true species difference or only due to differences in methodology. One possible way to do this would be to use monoclonal antibodies to isolate the different domains of AFP. The binding characteristics of each domain could then be studied without interference from the other.

The accumulated data on the ability of AFP to bind ligands suggest that the biological function of AFP as a carrier protein is similar but not identical to that of albumin. AFP might carry substances of special importance for the developing fetus that albumin does not carry. The binding of estrogen to rat and mouse AFP could exemplify this.

It is also possible as speculated by Ingvarsson (1979) that substances carried by albumin in the maternal circulation would be transferred to AFP in the fetal circulation. If AFP has a higher affinity for the substance such a transfer might be facilitated and the fetal supply of metabolites would be ensured. Likewise bilirubin has a lower affinity for AFP than it has for albumin (Ruoslahti et al., 1979). This might facilitate the clearance of this catabolite from the fetal circulation especially under circumstances when the concentration of AFP is much higher than the concentration of albumin as has been reported for the early fetal stages of the pig (Ingvarsson et al., 1978; Stone, 1981).

The differences in binding characteristics and in the developmental patterns of serum-AFP levels found between different species suggest that the function of AFP might vary between species or that the functions carried out by AFP are required at different developmental stages in different species.

MONOCLONAL ANTIBODIES TO AFP

Through the development of the monoclonal antibody technique (Köhler and Milstein, 1975; 1976) scientists have access to a powerful tool that can be used in analysis and preparation of antigens. Indeed monoclonal antibodies have proved to be extremely useful in submolecular analysis and delineation of antigenic determinants of macromolecules (Atherton and Hynes, 1981; Borrebaeck and Etzler, 1981; Christmann and Dahmus, 1981). Monoclonal antibodies can also be used to distinguish between genetic variants of proteins. Staehelin et al. (1981) described

a set of monoclonal antibodies to human leukocyte interferons that can be used as probes into the structure of this group of biologically active molecules.

Monoclonal antibodies to AFP have been described in several reports. Tsung et al. (1980) reported on the production of a monoclonal antibody with high affinity for human AFP. Uotila et al. (1980) described monoclonal antibodies to human AFP that can be used in highly sensitive radioimmunoassays and Uotila et al. (1981) developed a two-site enzyme-linked immunoassay with use of monoclonal antibodies to human AFP. In this latter assay the authors utilized the difference in specificity for antigenic determinants in the AFP molecule between two monoclonal antibodies. One monoclonal antibody was immobilized on the solid phase and the other labeled with enzyme. The sensitivity of the assay was very good. The use of monoclonal antibodies ensures the specificity of the assay and a large supply of identical reagents. In an attempt to obtain autoreactive monoclonal antibodies to mouse AFP we immunized mice of the Balb/C strain with rat AFP. The monoclonals so far obtained only react with rat AFP. They are of low affinity and are excellent for immunosorbent purposes (R. Carlsson, A.-C. Carlsson, E. Ruoslahti to be published).

Animals are immunologically tolerant to their own AFP. This tolerance can, however, be abrogated by immunization with chemically modified homologous AFP (Ruoslahti et al., 1975) or immunologically related heterologous AFP (Engvall et al., 1977; Ruoslahti and Wigzell, 1975). The resulting antibodies merely crossreact with the autologous AFP and are not real autoantibodies. Such a way to raise crossreactive antibodies ought to be possible to utilize for production of monoclonal antibodies that show autoantibody like reactivity. Such antibodies might prove especially advantageous for immunolocalization and immunotherapy of AFP-producing tumors as no immune reaction by the host against the administered antibody probe would occur. A disadvantage might be that antibodies produced in the way described above seem to be of low affinity (Jalanko et al., 1978a). For the human situation a possibility to get crossreactive autoantibodies would be to perform in vitro immuni-

zations of human lymphocytes with chemically modified or immunologically related antigens followed by hybridization with human myeloma cells (Olsson and Kaplan, 1980).

SYNTHESIS OF ALPHA-FETOPROTEIN

During normal development

During ontogenetic development alpha-fetoprotein is mainly synthesized by the fetal liver and yolk sac (Albrechtsen and Nørgaard-Pedersen, 1978; Gitlin and Boesman, 1967; Gitlin and Perricelli, 1970; paper V). A low rate of synthesis has also been detected in cells of the gastrointestinal tract (Gitlin et al., 1972) and kidney (Breborrowicz and Nishi, 1979). Other fetal organs, e.g. the brain, have been reported to contain AFP intracellularly (Benno and Williams, 1978; Mackiewicz et al., 1978). This is due to a leakage from blood and interstitium rather than to a local synthesis (Breborrowicz and Nishi, 1979).

The cells responsible for the production of AFP in the yolk sac are the visceral endodermal cells and in the liver the hepatocytes (Dziadek and Adamson, 1978; Dziadek, 1978; Gitlin and Boesman, 1967; Sell et al., 1975; paper V). Through immunofluorescence it has been shown that a single hepatocyte can simultaneously produce alpha-fetoprotein and albumin independently of each other (Nayak and Mital, 1977; Gleiberman, 1978; paper IV).

This confirmed earlier findings by Eraizer et al. (1977) who in a plaque assay showed that a single isolated hepatocyte could produce either of or both of AFP and albumin. It should be noted that none of the assays mentioned above measures production of AFP but rather measures cellular content of AFP. However, the interpretation that the AFP found in hepatocytes was actually produced there is justified under the condition that leakage of AFP from extracellular sources was controlled for and ruled out (Engelhardt et al., 1971; paper V).

The origin and fate of the AFP producing liver cell has been investigated. It is clear that the fetal hepatocytes produce AFP and that the adult ones do not, at least not to an appreciable amount. Results presented in paper V suggest strongly that the AFP producing fetal hepatocytes develop into adult hepatocytes which do not produce AFP. This concept is supported by findings from other laboratories. Engelhardt et al. (1976) and Watanabe et al. (1976) showed that adult mature hepatocytes could resume AFP-synthesis after appropriate stimulation and Perova et al. (1977) showed that fetal hepatocytes in culture could cease their production of AFP. That isolated adult hepatocytes can proliferate and produce AFP in vitro has been reported by Leffert et al. (1978). However, in this study the investigators did not show that the cells producing AFP originated from the mature hepatocytes. Recently, Kuhlmann (1979a) utilizing immuno-electron microscopy showed that AFP is produced by hepatocytes at all developmental stages from fetal to adult type during ontogeny.

Together all these results form evidence that indicate that production of AFP under nonmalignant conditions is a capability hepatocytes possess transiently during a certain stage of their development. Given the appropriate stimuli mature differentiated hepatocytes will resume production of AFP.

In cancer and nonmalignant liver injury

AFP is normally expressed by adult individuals at a low basal rate resulting in serum levels of AFP ranging from a few to a couple of hundred nanograms per milliliter depending on the species (Ruoslahti et al., 1974a). Elevations in the concentration of AFP are seen in individuals suffering from primary cancer of the liver and teratocarcinoma (Abelev, 1971; McIntire et al., 1976). More rarely elevations of the AFP levels are also associated with cancers of the gastrointestinal tract (McIntire et al., 1975; Ravry et al., 1974). Detection and determination of abnormal amounts of AFP in serum have proved a useful clinical tool for the early diagnosis and subsequent prognosis of the

malignancies described above (Ruoslahti et al., 1974b; McIntire et al., 1975, 1976; Okuda et al., 1975).

The exact cellular origin of the AFP-producing hepatoma is not known. The hepatoma cells themselves produce AFP (Abelev, 1971; Goussev et al., 1971; Kuhlmann, 1978a, b; Sell, 1978; Sell and Skelly, 1976) but hepatomas that do not produce AFP also exist (Ruoslahti et al., 1972, 1974b; Sell, 1978). This lack of 100% positive correlation between AFP and hepatoma is disappointing from a clinical point of view. The majority (70 - 80 %) of the hepatomas produce enough AFP to elevate the serum levels significantly (Alpert and Coston, 1975; Chen and Sung, 1972; McIntire et al., 1975; Wepsic and Sell, 1974).

When laboratory animals are treated with hepatocarcinogens, such as acetyl amino fluorene, N-nitrosomorpholine or 3-methyl-4-dimethyl-aminoazobenzene an early, transient elevation of AFP in serum is seen (Becker et al., 1975; Kitagawa et al., 1972; Ono et al., 1973; Watabe et al., 1971). This is also evident prior to the spontaneous development of primary hepatoma in old C3H-mice (Jalanko et al., 1978b). The elevations occur before any malignantly transformed cells can be observed but concomitantly with a heavy proliferation of the so called oval cells and transitional cells (Inoka, 1967; Iwasaki et al., 1972). These cells have been shown to synthesise AFP and to be responsible for the early elevation of the AFP levels (Dempo et al., 1975; Kuhlmann, 1978a; Tchipyshcheva et al., 1977). Stimulation of proliferation of the oval cells and activation of the AFP gene have been interpreted to be the first step in a chain of events ultimately leading to the formation of a hepatoma (Inaoka, 1967). The idea that hepatomas exclusively originate from the oval cells has recently been challenged by Becker and Sell (1979) and Sell et al. (1980). Administration of some other hepatocarcinogens e.g. diethylnitrosamine (DEN) or low dosages of N-nitrosomorpholine will not produce any early elevation of serum-AFP levels and no proliferation of oval cells. With DEN as carcinogen AFP is first detected at the stage when premalignant lesions occur, and no connection between the oval cell and hepatoma can be observed (Sell et al., 1980).

Depending on the carcinogen other cell types than the oval cells, namely the mature hepatocytes, could serve as target cells for the carcinogen and produce the hepatoma (Sell et al., 1980). The model gives a possible explanation for the existence of AFP positive and AFP negative hepatomas. The hepatomas that produce AFP originate from the oval cells and atypical hyperplasia and the hepatomas that do not produce AFP originate from AFP-negative preneoplastic nodules.

AFP can also be expressed in adult individuals as a response to liver injury. The liver injury can be experimentally induced by e.g. partial hepatectomy, administration of hepatotoxins, such as carbon-tetrachloride, ethionine and galactosamine (Abelev et al., 1963; Engelhardt et al., 1976; Sell et al., 1974; Smuckler et al., 1976; Watanabe et al., 1976) or can be caused by nonmalignant liver disease, liver cirrhosis and hepatitis (Abelev, 1971; Alexander et al., 1978; Ruoslahti et al., 1974b). A close relationship between proliferation of malignant and nonmalignant liver cells and production of AFP has been established both in vivo (Guillouzo et al., 1979; Leffert and Sell, 1974) and in vitro (Leffert et al., 1978; Sell et al., 1975; Tsukada and Hirai, 1975, 1976; Watabe et al., 1976). There is no obligatory link between proliferation of liver cells and production of AFP, as liver cells can proliferate without AFP-production (Smuckler et al., 1976) and AFP can be produced without proliferation (Watanabe et al., 1976). Thus other factors than rate of proliferation must also influence the expression of the AFP gene.

CONTROL OF AFP SYNTHESIS

A number of investigations have shown that the synthesis of AFP is controlled at the transcriptional level. The amount of synthesized AFP correlates to the amount of messenger RNA for AFP during normal ontogenetic development (Chiu et al., 1979; Innis and Miller, 1977; Koga et al., 1974; Liao et al., 1980b; Sala-Trepas et al., 1979a), in association with regeneration of liver after liver damage (Chiu et al., 1981; Sell et al., 1980) and in hepatomas (Innis and Miller,

1977, 1979; Sala-Trepat et al., 1979a). In addition, the number of genes and the gene organization are similar for AFP and albumin. Probably only one single gene copy exists per haploid genome and both the AFP and the albumin gene are similarly organized with more than 10 intervening sequences (Gorin and Tilghman, 1980; Liao et al., 1980; Sala-Trepat et al., 1979b).

The exact molecular events that govern the turning on and off of the AFP gene are not understood. A new and interesting approach to this problem was recently taken by Szpirer et al. (1980). Through somatic cell hybridization of an AFP-producing mouse hepatoma with isolated rat hepatocytes they tried to activate the quiescent rat AFP gene. Strikingly, in none of the resulting hybrids was rat AFP expressed though mouse AFP was.

The molecular mechanisms responsible for keeping the mouse AFP gene active could not activate the rat AFP gene and the silent rat AFP gene did not cause extinction of the expression of the mouse AFP gene. The authors concluded that the activation of the AFP gene in the developing hepatocyte is probably under cis-control and could not be transferred from one chromosome to another. Similar results have been reported by Cassio and Weiss (1979). However, when AFP-producing hepatoma cells are fused with nonepithelial cells e.g. fibroblasts a total extinction of the AFP gene occurs. This implies that cells that never produce AFP during ontogenetic development possess a factor(s) that can inactivate the AFP gene and that the regulation of the AFP gene is different in e.g. hepatocytes and fibroblasts (Szpirer and Szpirer, 1975; 1979).

Synthesis of AFP can be influenced by a variety of hormones both in vitro and in vivo. Belanger et al. (1975) reported that injections of glucocorticoids suppressed the production of AFP in neonatal rats and mice. Glucocorticoids given to adult carbon-tetrachloride poisoned animals, on the contrary, enhanced the production of AFP (Konyaeva and Vishnevetskii, 1977). That the decreased serum AFP levels after dexamethasone administration to newborn mice was in fact caused by a

repression of the AFP gene was shown by Commer et al. (1979) who found that the mRNA levels for AFP showed a proportional decrease. Dexamethasone administered in vitro enhances the production of AFP by hepatoma cells that already produce it (Tsukada et al. 1979) but does not induce expression of AFP in hepatoma cells that do not produce AFP (Yoo et al., 1979). Freeman et al. (1981) found that hydrocortisone was essential for the maintenance of production of AFP by longterm cultures of fetal mouse hepatocytes.

The reports cited above demonstrate that hormones may influence the expression of the AFP gene. The conflicting results on the action of a certain hormone makes it unlikely that hormones are the sole regulators of AFP-synthesis in vivo.

Tumyan et al. (1975) and Carlsson and Ingvarsson (1977) proposed that factors present in adult serum could inhibit AFP-synthesis. Injections of adult serum into neonates accelerated the decrease in serum AFP levels. I have recently undertaken a study in which adult mouse serum, treated in various ways, was injected into newborn mice following the procedure of Tumyan et al. (1975). The results of this investigation is presented in Table II. Irrespective of treatment of the serum the injections always gave approximately the same reduction in AFP levels. The degree of reduction was generally lower than that reported by Tumyan et al. (1975) and correlated to the injected amount of protein. It therefore seems that the reduction in serum AFP levels obtained by injections of adult serum is unspecific and could just as well depend on an increase in catabolism as on a decrease in synthesis.

Production of AFP appears to be under regulation of a single Mendelian gene, the Raf gene (Lindahl et al., 1978; Ohlsson et al., 1977). This gene is expressed in normal Balb/CJ mice rendering them a persisting, high level of AFP during neonatal development and adulthood. The same gene regulates the reexpression of AFP in adults in association with liver injury (Jalanko, 1979; Lindahl et al., 1978). Kuhlmann (1979b) showed by use of immunoperoxidase technique that normal adult liver from Balb/CJ mice but not from C3H mice contained detectable amounts

Table II. Effect of injections of adult mouse serum on the serum levels of AFP in neonatal mice.

Injected material	Levels of AFP ^a , per cent of controls ^b
Phosphate buffered saline	74.2 $\pm$ 12.6 SEM ^c
Normal mouse serum	51.8 $\pm$ 10.3 SEM ^d
Normal mouse serum 1/4 volume	70; 85
Normal mouse serum 1/10 volume	78; 92
Normal mouse serum 1/100 volume	94; 103
Dialyzed mouse serum	40; 58
TCA precipitate of mouse serum	51; 59
Frozen-thawed mouse serum (5 times)	43; 52
Heat inactivated mouse serum (60°C, 45 min)	40; 70
Serum from hepatectomized mice	50; 53
Supernatant of 40% ammonium sulfate precipitated mouse serum	50; 72
Precipitate of 40% ammonium sulfate precipitated mouse serum	67; 89

a) Each value represents the mean of four individuals within the same litter.

b) Controls were injected with phosphate buffered saline.

c and d) Mean of 6 litters. Note that the value under c is expressed as percentage of levels in uninjected animals.

Neonatal mice were injected once a day for nine days following the schedule of Tumyan et al. (1975) with substances indicated in the table. The animals were killed on the 10th day and the concentrations of AFP were measured by the Laurell rocket technique (1966). Monospecific anti-mouse AFP was kindly provided by Dr. G. Lindahl, Dep. of Med. Microbiol. Lund. The serum from hepatectomized mice were collected 48 hrs after partial hepatectomy. TCA and ammonium sulfate precipitates of mouse serum were dissolved and dialysed against phosphate buffered saline as was the supernatant from the ammonium sulfate precipitation. The dissolved precipitates were then adjusted to the original volume. The concentration of protein was 32 mg/ml in the supernatant and 15 mg/ml in the dissolved ammonium sulfate precipitate.

of AFP. The cells containing AFP were mainly localized around the central veins. After partial hepatectomy a very large proportion of the hepatocytes were positive for AFP. My studies have confirmed this (unpublished data). It is interesting that the cells that contain AFP in the adult

Balb/CJ mouse are found abutting the central vein. Cells with the same localization are the last ones to loose the production of AFP during neonatal development (Engelhardt et al., 1974; Shipova et al., 1974; paper V). This indicates that the proximity to the central vein or the distance from the periphery of the liver lobule is of importance for a sustained production of AFP.

AFP in hepatocytes dissappears progressively from the periphery of the liver lobule towards the central vein during the neonatal development (Engelhardt et al., 1974; Nayak and Mital, 1977; Stratil et al., 1976; paper V). This might be caused by a progressive change in the molecular composition of the cell matrix starting at the periphery of the liver lobule. Influence on gene expression by cell matrices and cell interactions have been reported in other systems (Grobstein, 1975). Indeed Dziadek (1978) found that production of AFP by the visceral endoderm of the yolk sac was inhibited by direct contact with extra embryonic ectoderm. Abelev (1979) and Engelhardt et al. (1979) have shown that changes in distribution and expression of a liver antigen called Ag-I or the bile canaliculi antigen occurs concomitantly but inversely with changes in expression of AFP in livers from neonates and carbontetrachloride poisoned adult mice. The authors hypothesize that the organisation of the definite structure of the liver cord is responsible for the neonatal disappearance of AFP production. Similarly the disorganization of the stable structure occuring after partial hepatectomy or chemically induced liver injury would cause the reexpression of AFP production (Abelev, 1978). On a molecular level this must mean that the composition of the environment in the immediate vicinity of the hepatocytes must change or that the ability of the hepatocytes to interact with the environment must change when the expression of the AFP gene is repressed or derepressed.

In paper VI we have shown that during a regenerative response of the liver induced by carbon-tetrachloride poisoning or partial hepatectomy, changes in the composition of the cell matrix and in the ability of the hepatocytes to interact with matrix components especially laminin occur. Laminin is a large glycoprotein normally found in basement membranes (Foidart et al., 1980; R. Carlsson, E. Hayman and E. Ruoslahti, to be published). It has

cellattachment functions (Terranova et al., 1980; Vlodavsky and Godspodarowicz, 1981; paper VI). The changes in expression of laminin and in the ability of the hepatocytes to interact with laminin coincide with the permissive state of AFP-synthesis. The increased amount of laminin localized to the perinecrotic and postnecrotic areas of the liver lobule where AFP is synthesized after carbon-tetrachloride poisoning (Engelhardt et al., 1976; Mohanty et al., 1978). There is no evidence as yet that there exists a direct link between production of AFP and laminin. It is possible that the synthesis of AFP and the changes in laminin are both caused by the disorganization of the liver cord after the necrotic damage and that they occur concomitantly but independently of each other.

The results from paper VI have given a possible molecular basis for further studies concerning influences of the cell matrix on gene regulation in general and on the regulation of the AFP gene in particular. The effect of purified matrix components on the expression of the AFP gene could be studied by allowing hepatocytes at different developmental stages to be cultured on isolated defined matrix components and on mixtures of these components.

SUMMARY AND CONCLUDING REMARKS

Alpha-fetoprotein (AFP) from fetal swine serum was isolated and characterized physicochemically regarding molecular weight, sedimentation coefficient, isoelectric point and amino acid composition. AFP was found to exhibit similarities with AFPs from other species and with isolated porcine albumin.

Porcine and bovine AFP were shown to bind fatty acids. Isolated bovine AFP was found to contain a variety of fatty acids as did fetal bovine albumin. The quality of the bound fatty acids differed between AFP and albumin. AFP bound more long-chain polyunsaturated fatty acids than albumin. Bovine AFP and probably porcine AFP were shown to have an especially high affinity for arachidonic acid.

By the aid of immunofluorescence technique AFP could be detected in all hepatocytes, but not in hematopoietic cells, of the fetal and neonatal pig liver during the first five days after birth. After the fifth day AFP-negative hepatocytes appeared at the periphery of the lobule. No AFP-containing cells could be detected in the adult liver. Albumin could only be detected in a small proportion of the hepatocytes in the early fetal liver. The proportion increased with increasing gestational age and after 70% of gestation all hepatocytes contained albumin. The dynamics in staining intensity and in number of cells that contained AFP or albumin corresponded well to changes in serum levels of the two proteins.

During conditions that are permissive for synthesis of AFP in the adult nonmalignant liver, changes occur in the expression and distribution of certain matrix components i.e. laminin and to some extent fibronectin. In addition, the ability of hepatocytes to interact with these matrix components changes during the course of the regenerative response of the liver. Such changes in the molecular composition and in the ability of the liver cells to interact with specific matrix components might have a direct or indirect regulatory influence on the synthesis of AFP.

The molecular similarities between AFP and albumin recently unveiled

suggest that AFP could function as a fetal counterpart to albumin. However, it may be wrong to regard AFP as just a fetal albumin. The functions of the two proteins, as extrapolated from their in vitro binding capabilities, are similar but not identical. Differences in affinity for ligands exist between the two proteins and some properties are known to be specific for one of the proteins e.g. the binding of estrogen to rodent AFP.

The changes in serum levels of AFP during the lifespan of an individual can be very dramatic. The levels are high during fetal life, very low during normal adult life and are elevated in adults suffering from certain types of carcinomas or nonmalignant liver disease that lead to proliferation of hepatocytes. Such spectacular changes in the expression of an antigen give great incentives to use this antigen for studies on gene regulation. Although no directly regulatory system for AFP has been discerned as yet I think that the recent findings on variation in expression of antigens of the liver and liver cell functions in permissive states of AFP-synthesis show promise. Further studies on a regulatory role of liver structure or of cell interactions or cell-matrix interactions might enable us to better understand how the synthesis of AFP is regulated and how mammalian genes in general are regulated.

ACKNOWLEDGEMENTS

This work has been carried out at the Department of Zoophysiology, University of Lund, Lund, Sweden and of the La Jolla Cancer Research Foundation, La Jolla, California, USA.

I wish to express my deep gratitude to colleagues and friends at the Department of Zoophysiology and at the La Jolla Cancer Research Foundation for inspiring discussions and collaborations. I am especially indebted to Drs Börje Karlsson, Eva Engvall and Erkki Ruoslahti for their constructive criticism and support during the course of this work.

I also wish to thank Mrs. Elisabeth Andersson and Miss Marianne Andersson for skilful secretarial assistance.

Financial support has been received from the Royal Physiographical Society of Lund, The Expressen Prenatal Research Foundation, The Harald and Greta Jeansson Foundation and the National Institutes of Health.

REFERENCES

- Abelev, G.I. (1971) Adv. Cancer Res. 14, 295-358.
- Abelev, G.I. (1978) In: Cell Differentiation and Neoplasia, (Saunders, G.G. ed.) Raven Press, New York pp. 257-269.
- Abelev, G.I. (1979) In: Carcino-Embryonic Proteins, Vol 1. (Lehmann, F.-G. ed.) pp. 99-110.
- Abelev, G.I., Perova, S.O., Khramkova, N.I., Postnikova, Z.A. and Irlin, I.S. (1963) Transplantation 1, 174-180.
- Albrechtsen, R. and Nørgaard-Pedersen, B. (1978) Scand J. Immunol. 8, Suppl. 8, 193-199.
- Alexander, M.G., Purves, L.R., Kirsch, R.E., Bass, N.M., Gitlin, N., Terblanche, J. and Saunders, S.J. (1978) S. Afr. med. J. 53, 433-436.
- Aliau, S., Marti, J. and Moretti, J. (1978) Biochimie 60, 663-672.
- Alpert, E. and Coston, R.L. (1975) Clin. Chim. Acta 58, 77-83.
- Alpert, E. and Perencevich, R.C. (1975) Ann. N.Y. Acad. Sci. 259, 131-135.
- Alpert, E., Drysdale, J.W., Isselbacher, K.J. and Schur, P.H. (1972) J. Biol. Chem. 247, 3792-3798.
- Atherton, B.T. and Hynes, R.O. (1981) Cell 25, 133-141.
- Attardi, B. and Ruoslahti, E. (1977) Steroids 30, 711-716.
- Aussel, C. and Masseyeff, R. (1978) J. Steroid. Biochem. 9, 547-551.
- Aussel, C., Uriel, J. and Mercier-Bodard, C. (1973) Biochimie 55, 1431-1437.
- Barus, M., Sylwester, D., Kelleher, P.C. and Smith, C.J. (1978) J. Natl. Cancer Inst. 61, 833-836.
- Bayard, B. and Kerckaert, J.-P. (1977) Biochim. Biophys. Res. Commun. 77, 489-495.
- Becker, F.F. and Sell, S. (1979) Cancer Res. 39, 1437-1442.
- Becker, F.F., Horland, A.A., Shurgin, A. and Sell, S. (1975) Cancer Res. 35, 1510-1513.
- Belanger, L., Hamel, D., Lachance, L., Dufour, D., Tremblay, M. and Gagnon, P.M. (1975) Nature 256, 657-659.
- Benassayag, C., Savu, L., Vallette, G., Delorme, J. and Nunez, E.A. (1979) Biochim. Biophys. Acta 587, 227-237.
- Benassayag, C., Vallette, G., Delorme, J., Savu, L. and Nunez, E.A. (1980) Oncodev. Biol. Med. 1, 27-36.
- Benno, R.H. and Williams, T.H. (1978) Brain Res. 142, 182-186.

- Berde, C.B., Nagai, M. and Deutsch, H.F. (1979) J. Biol. Chem. 254, 12609-12614.
- Bergstrand, C. and Czar, B. (1956) Scand. J. Clin. lab. Invest 8, 174.
- Borrebaeck, C.A.K. and Etzler, M.E. (1981) J. Biol. Chem. 256, 4723-4725.
- Branch, W.R. (1972) Int. J. Cancer 10, 451-457.
- Breborowicz, J. and Nishi, S. (1979) In: Carcino-Embryonic Proteins Vol. II (Lehmann, F.-G. ed.) pp. 265-270.
- Bøg-Hansen, T.C., Bjerrum, O.J. and Ramlau, J. (1975) Scand. J. Immunol. 4, Suppl. 2, 141-147.
- Carlsson, R.N.K. and Ingvarsson, B.I. (1977) Biol. Neonate 32, 183-188.
- Cassio, D. and Weiss, M.C. (1979) Somat. Cell Genet. 5, 719-738.
- Chen, D.-S. and Sung, J.-L. (1977) Cancer 40, 779-783.
- Chiu, J.-F., Decha-Umphai, W. and Commer, P. (1979) Nucleic Acid Res. 7, 239-249.
- Chiu, J.-F., Gabryelak, T., Commer, P. and Massari, R. (1981) Biochim. Biophys. Res. Commun. 98, 250-254.
- Christmann, J.L. and Dahmus, M.E. (1981) Fed. Proc. Abstr. 40, 1736.
- Commer, P., Schwartz, C., Tracy, S., Tamaoki, T. and Chiu, J.-F. (1979) Biochim. Biophys. Res. Commun. 89, 1294-1299.
- Davidson, E.H. (1976) Gene activity in Early Development, 2nd edn., Academic Press, New York, pp. 1-26.
- Dempo, K., Chisaka, N., Yoshida, Y., Kaneko, A. and Onoé, T. (1975) Cancer Res. 35, 1282-1287.
- Dziadek, M. (1978) J. Embryol. exp. Morph. 46, 135-146.
- Dziadek, M. and Adamson, E. (1978) J. Embryol. exp. Morph. 43, 289-313.
- Endo, Y., Kanai, K., Iino, S. and Oda, T. (1974) In L'Alpha-Foetoproteine (Masseyeff, R. ed.) Colloque de L'INSERM, Nice, pp. 47-54.
- Engelhardt, N.V., Gleiberman, A.S., Lazareva, M.N. and Poltoranina, V.S. (1979) In: Carcino-Embryonic Proteins Vol. I (Lehmann, F.-G. ed.) pp. 111-119.
- Engelhardt, N.V., Goussev, A.I., Shipova, L.Y. and Abelev, G.I. (1971) Int. J. Cancer 7, 198-206.
- Engelhardt, N.V., Lazareva, M.N., Abelev, G.I., Uryvaeva, I.V., Factor, V.M. and Brodsky, V.Y. (1976) Nature 263, 146-148.
- Engelhardt, N.V., Poltoranina, V.S., Shipova, L.Y., Goussev, A.I. and Yazova, A.K. (1974) In: L'Alpha-Foetoproteine (Masseyeff, R. ed.) Colloque de L'INSERM, Nice, pp. 217-229.

- Engvall, E., Pihko, H., Jalanko, H. and Ruoslahti, E. (1977) J. Natl. Cancer Inst. 59, 277-280.
- Eraizer, T.L., Elgort, D.A. and Abelev, G.I. (1977) Bull. Exp. Biol. Med. 83, 843-846.
- Foidart, J.M., Bere, E.W., Yaar, M., Rennard, S.I., Gullino, M., Martin, G.R. and Katz, S.I. (1980) Lab. Invest. 42, 336-342.
- Freeman, A.F., Engvall, E., Hirata, K., Yoshida, Y., Kottel, R.H., Hilborn, V. and Ruoslahti, E. (1981) Proc. Natl. Acad. Sci. USA, 78, 3659-3663.
- Gitlin, D. (1974) In: L⁻Alpha-Foetoproteine (Masseyeff, R. ed.) Colloque de L⁻INSERM, Nice, pp. 55-72.
- Gitlin, D. (1975) Ann. N.Y. Acad. Sci. 259, 7-16.
- Gitlin, D. and Boesman, M. (1967) Comp. Biochem. Physiol. 21, 327-336.
- Gitlin, D. and Perricelli, A. (1970) Nature 228, 995-997.
- Gitlin, D., Perricelli, A. and Gitlin, G.M. (1972) Cancer Res. 32, 979-982.
- Gleiberman, A.S. (1978) Bull. Exp. Biol. Med. 85, 689-693.
- Gorin, M.B. and Tilghman, S.M. (1980) Proc. Natl. Acad. Sci. USA 77, 1351-1355.
- Goussev, A.I., Engelhardt, N.V., Masseyeff, R. and Basteris, B. (1971) Int. J. Cancer 7, 207-217.
- Grigorova, A.-M., Cittanova, N. and Jayle, M.-F. (1977) Biochimie 59, 217-220.
- Grobstein, C. (1975) In: Extracellular Matrix Influences on Gene Expression (Slavkin, H.C. and Grenlich, R.C. eds.) Academic Press, New York, pp. 9-16.
- Guillouzo, A., Boissnard-Rissel, M., Belanger, L. and Bourel, M. (1979) Biochim. Biophys. Res. Commun. 91, 327-331.
- Hassoux, R., Poupon, M.F. and Uriel, J. (1978) Ann. Immunol. (Inst. Pasteur) 129 C, 275-285.
- Hsia, J.C., Er, S.S., Tan, C.T., Estes, T. and Ruoslahti, E. (1980) J. Biol. Chem. 255, 4224-4227.
- Inaoka, Y. (1967) GANN 58, 355-366.
- Ingvarsson, B. (1979) Thesis, Lund.
- Ingvarsson, B.J., Carlsson, R.N.K. and Karlsson, B.W. (1978) Biol. Neonate 34, 259-268.

- Innis, M.A. and Miller, D.L. (1977) J. Biol. Chem. 252, 8469-8475.
- Innis, M.A. and Miller, D.L. (1979) J. Biol. Chem. 254, 9148-9154.
- Innis, M.A. and Miller, D.L. (1980) J. Biol. Chem. 255, 8994-8996.
- Iwasaki, T., Dempo, K., Kaneko, A. and Onoé, T. (1972) GANN 63, 21-30.
- Jagodzinski, L.J., Sargent, T.D., Yang, M., Glackin, C. and Bonner, J. (1981) Proc. Natl. Acad. Sci. USA 78, 3521-3525.
- Jalanko, H. (1979) Int. J. Cancer 24, 394-397.
- Jalanko, H., Engvall, E. and Ruoslahti, E. (1978a) Immunol. Commun. 7, 209-222.
- Jalanko, H., Virtanen, J., Engvall, E. and Ruoslahti, E. (1978b) Int. J. Cancer 21, 453-459.
- Karlsson, B.W. (1970) Comp. Biochem. Physiol. 34, 535-546.
- Karlsson, B.W. (1972) Life Sci. 11, 169-176.
- Kekomäki, M., Seppälä, M., Ehnholm, C., Schwartz, A.L. and Raivio, K. (1971) Int. J. Cancer 8, 250-258.
- Keller, R.H., Calvanico, N.J. and Tomasi, T.B. (1976) In: Onco-Developmental Gene Expression (Fishman, W.H. and Sell, S. eds.) Academic Press, New York, pp. 287-299.
- Kerckaert, J.-P., Bayard, B. and Biserte, G. (1979) Biochim. Biophys. Acta 576, 99-108.
- Kitagawa, T., Yokochi, T. and Sugano, H. (1972) Int. J. Cancer 10, 368-381.
- Kithier, K. and Poulik, M.D. (1972) Biochim. Biophys. Acta 278, 505-516.
- Koga, K., O'Keefe, D.W., Iio, T. and Tamaoki, T. (1974) Nature 252, 495-497.
- Konyaeva, A.G. and Vishnevetskii, F.E. (1977) Bull. Exp. Biol. Med. 83, 146-148.
- Kuhlmann, W.D. (1978a) Int. J. Cancer 21, 368-380.
- Kuhlmann, W.D. (1978b) Int. J. Cancer 22, 335-343.
- Kuhlmann, W.D. (1979a) J. Ultrastruct. Res. 68, 109-117.
- Kuhlmann, W.D. (1979b) Histochemistry 64, 67-75.
- Köhler, G. and Milstein, C. (1975) Nature (Lond.) 256, 495-497.
- Köhler, G. and Milstein, C. (1976) Eur. J. Immunol. 6, 511-519.
- Lai, P.C.W., Hay, D.M., Peters, E.H. and Lorscheider, F.L. (1977) Biochim. Biophys. Acta 493, 201-209.
- Laurell, C.-B. (1966) Analyt. Biochem. 15, 45-52.

- Law, S.W. and Dugaiczky, A. (1981) *Nature* 291, 201-205.
- Leffert, H.L. and Sell, S. (1974) *J. Cell. Biol.* 61, 823-829.
- Leffert, H.L., Moran, T., Sell, S., Skelly, K., Ibsen, K., Mueller, M. and Arias, I. (1978) *Proc. Natl. Acad. Sci. USA* 75, 1834-1838.
- Lester, E.P., Miller, J.B. and Yachnin, S. (1978a) *Immunol. Commun.* 7, 137-161.
- Lester, E.P., Miller, J.B., Baron, J.M. and Yachnin, S. (1978b) *Immunology* 34, 189-198.
- Leung, C.C.K., Watabe, H. and Brent, R.L. (1977) *Am. J. Anat.* 148, 457-462.
- Liao, W.S., Hamilton, R.W. and Taylor, J.M. (1980a) *J. Biol. Chem.* 255, 8046-8049.
- Liao, W.S.L., Conn, A.R. and Taylor, J.M. (1980b) *J. Biol. Chem.* 255, 10036-10039.
- Lindahl, G., Olsson, M. and Ruoslahti, E. (1978) *Scand. J. Immunol.* 8, Suppl. 8, 209-212.
- Littman, B.H., Alpert, E. and Rocklin, R.E. (1977) *Cell. Immunol.* 30, 35-42.
- Mackiewicz, A., Hejduk, W. and Breborowicz, J. (1978) *Scand. J. Immunol.* 8, Suppl. 8, 231-238.
- McIntire, K.R., Waldmann, T.A., Moertel, C.G. and Go, V.L.W. (1975) *Cancer Res.* 35, 991-996.
- McIntire, K.R., Vogel, C.L., Primack, A., Waldmann, T.A. and Kyalwazi, S.K. (1976) *Cancer* 37, 677-683.
- Mizejewski, G.J. and Grimley, P.M. (1976) *Nature* 259, 222-224.
- Mohanty, M., Das, P.K., Mittal, A. and Nayak, N.C. (1978) *Int. J. Cancer* 22, 181-188.
- Murgita, R.A. and Tomasi, T.B. (1975a) *J. Exp. Med.* 141, 269-286.
- Murgita, R.A. and Tomasi, T.B. (1975b) *J. Exp. Med.* 141, 440-452.
- Murgita, R.A. and Wigzell, H. (1976) *Scand. J. Immunol.* 5, 1215-1220.
- Murgita, R.A., Andersson, L.C., Sherman, M.S., Bennich, H. and Wigzell, H. (1978) *Clin. Exp. Immunol.* 33, 347-356.
- Murgita, R.A., Goidl, E.A., Kontiainen, S., Beverly, P.C.L. and Wigzell, H. (1978) *Proc. Natl. Acad. Sci. USA* 75, 2897-2901.
- Murgita, R.A., Goidl, E.A., Kontiainen, S. and Wigzell, H. (1977) *Nature* 267, 257-259.

- Nayak, N.C. and Mital, I. (1977) Amer. J. Pathol. 86, 359-374.
- Nishi, S. (1970) Cancer Res. 30, 2507-2513.
- Nishi, S. and Hirai, H. (1972) Biochim. Biophys. Acta 278, 293-298.
- Nishi, S., Katsuno, Y. and Hirai, H. (1976) In: Onco-Developmental Gene Expression (Fishman, W.H. and Sell, S. eds.) Academic Press, New York, pp. 679-683.
- Nishi, S., Watabe, H. and Hirai, H. (1975) Ann. N.Y. Acad. Sci. 259, 109-118.
- Nørgaard-Pedersen, B., Toftager-Larsen, K., Philip, J. and Hindersen, P. (1980) Clin. Genet. 17, 355-362.
- Okuda, K., Kotoda, K., Obata, H., Hayashi, N., Hisamitsu, T., Tamiya, M., Kubo, Y., Yakushiji, F., Nagata, E., Jihouchi, S. and Shimokawa, Y. (1975) Gastroenterology 69, 226-234.
- Olsson, L. and Kaplan, H.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5429-5431.
- Olsson, M., Lindahl, G. and Ruoslahti, E. (1977) J. Exp. Med. 145, 819-827.
- Onoé, T., Dempo, K., Kaneko, A. and Watabe, H. (1973) GANN Monograph on Cancer Res. 14, 233-247.
- Page, M. (1973) Can. J. Biochem. 51, 1213-1215.
- Parker, C.W., Kelly, J.P., Falkenheim, S.F. and Huber, M.G. (1979) J. Exp. Med. 149, 1487-1507.
- Parmelee, D.C., Evenson, M.A. and Deutsch, H.F. (1978) J. Biol. Chem. 253, 2114-2119.
- Parmely, M.J. and Hsu, H.F. (1973) Fed. Proc. (Abstr.) 32, 979.
- Peck, A.B., Murgita, R.A. and Wigzell, H. (1978) J. Exp. Med. 147, 667-683.
- Perova, S.D., Erazier, T.L. and Abelev, G.I. (1977) Bull. Exp. Biol. Med. 86, 569-572.
- Pihko, H. and Ruoslahti, E. (1973) Int. J. Cancer 12, 354-360.
- Pihko, H., Lindgren, J. and Ruoslahti, E. (1973) Immunochemistry 10, 381-385.
- Porath, J., Axén, R. and Ernback, S. (1967) Nature 215, 1491.
- Raftell, M. and Perlmann, P. (1969a) Expl. Cell Res. 49, 317-331.
- Raftell, M. and Perlmann, P. (1969b) Expl. Cell Res. 49, 332-339.
- Ravry, M., McIntire, R.K., Moertel, C.G., Waldmann, T.A., Schutt, A.J. and Go, V.L.W. (1974) J. Natl. Cancer Inst. 52, 1019-1021.

- Ren, E.C. and Chan, S.H. (1981) *J. Natl. Cancer Inst.* 66, 625-630.
- Ruoslahti, E., ed. (1976) *Scand. J. Immunol. Suppl.* 3, 39.
- Ruoslahti, E. (1978) *J. Immunol.* 121, 1687-1690.
- Ruoslahti, E. and Adamson, E. (1978) *Biochem. Biophys. Res. Commun.* 85, 1622-1630.
- Ruoslahti, E. and Engvall, E. (1976) *Proc. Natl. Acad. Sci. USA* 73, 4641-4644.
- Ruoslahti, E. and Engvall, E. (1978) *Scand. J. Immunol. Suppl.* 6, 1-17.
- Ruoslahti, E. and Seppälä, M. (1971) *Int. J. Cancer* 7, 218-225.
- Ruoslahti, E. and Seppälä, M. (1972) *Nature* 235, 161-162.
- Ruoslahti, E. and Terry, W.D. (1976) *Nature* 260, 804-805.
- Ruoslahti, E. and Wigzell, H. (1975) *Nature* 255, 716-717.
- Ruoslahti, E., Engvall, E., Pekkala, A. and Seppälä, M. (1978) *Int. J. Cancer* 22, 515-520.
- Ruoslahti, E., Estes, T. and Seppälä, M. (1979) *Biochem. Biophys. Acta* 578, 511-519.
- Ruoslahti, E., Pihko, H., Becker, M. and Mäkelä, O. (1975) *Eur. J. Immunol.* 5, 7-10.
- Ruoslahti, E., Pihko, H. and Seppälä, M. (1974a) *Transplant. Rev.* 20, 38-60.
- Ruoslahti, E., Salaspuro, M., Pihko, H., Andersson, L. and Seppälä, M. (1974b) *Br. Med. J.* 2, 527-529.
- Ruoslahti, E., Seppälä, M., Vuopio, P., Saksela, E. and Peltokallio, P. (1972) *J. Natl. Cancer Inst.* 49, 623.
- Sala-Trepat, J.M., Dever, J., Sargent, T.D., Thomas, K., Sell, S. and Bonner, J. (1979a) *Biochemistry* 18, 2167-2178.
- Sala-Trepat, J.M., Sargent, T.D., Sell, S. and Bonner, J. (1979b) *Proc. Natl. Acad. Sci. USA* 76, 695-699.
- Savu, L., Benassayag, C., Vallette, G., Nunez, E. and Jayle, M.-F. (1977) *Biochimie* 59, 323-328.
- Savu, L., Vallette, G., Nunez, E., Azria, M. and Jayle, M.-F. (1974) In: *L'Alpha-Foetoproteine* (Masseyeff, R. ed.) Colloque de L'INSERM, Nice, pp. 75-83.
- Sell, S. (1978) *Cancer Res.* 38, 3107-3113.
- Sell, S. and Skelly, K. (1976) *J. Natl. Cancer Inst.* 56, 645-648.
- Sell, S., Nichols, M., Becker, F.F. and Leffert, H.L. (1974) *Cancer Res.* 34, 865-871.

- Sell, S., Sala-Trepat, J.M., Sargent, T.D., Thomas, K., Nahon, J.-L., Goodman, T.A. and Bonner, J. (1980) *Cell Biol. Int. Rep.* 4, 235-254.
- Sell, S., Sheppard, H.W. and Poler, M. (1977) *J. Immunol.* 119, 98-103.
- Sell, S., Skelly, K., Leffert, H., Muller-Eberhard, U. and Kida, S. (1975) *Ann. N.Y. Acad. Sci.* 259, 45-58.
- Sheppard, H.W., Sell, S., Trefts, P. and Bahu, R. (1977) *J. Immunol.* 119, 91-97.
- Shipova, L.Y., Goussev, A.I. and Engelhardt, N.V. (1974) *Ontogenesis* 5, 53-60.
- Slade, B. (1973) *Nature* 246, 493.
- Smith, J.A. (1972) *Lancet*, April 15, 851.
- Smith, C.J. and Kelleher, P.C. (1973) *Biochim. Biophys. Acta* 317, 231-235.
- Smith, C.J., Kelleher, P.C., Belanger, L. and Dallaire, L. (1979) *Br. Med. J.* 1, 920-921.
- Smith, C.J., Morris, H.P. and Kelleher, P.C. (1977) *Cancer Res.* 37, 2651-2656.
- Smuckler, E.A., Koplitiz, M. and Sell, S. (1976) *Cancer Res.* 36, 4558-4561.
- Soloff, M., Swartz, S., Pearlmutter, A. and Kithier, K. (1976) *Biochim. Biophys. Acta* 427, 644-651.
- Sonnenschein, C. and Soto, A.M. (1979) *J. Natl. Cancer Inst.* 63, 835-841.
- Soto, A.M. and Sonnenschein, C. (1980) *Proc. Natl. Acad. Sci. USA* 77, 2084-2087.
- Soubiran, P., Mucchielli, A., Kerckaert, J.-P., Bayard, B. and Masseyeff, R. (1979) *Scand. J. Immunol.* 10, 179-186.
- Staehelin, T., Durrer, B., Schmidt, J., Takacs, B., Stocker, J., Miggiano, V. Stähli, C., Rubinstein, M., Levy, W.P., Hersherberg, R. and Pestka, S. (1981) *Proc. Natl. Acad. Sci. USA* 78, 1848-1852.
- Stone, R.T. (1981) *Biol. Reprod.* 24, 573-580.
- Stratil, P., Dolezalova, V., Feit, Y. and Kocent, A. (1976) *Neoplasma* 23, 1-10.
- Szpirer, J. and Szpirer, C. (1975) *Cell* 6, 53-60.
- Szpirer, J. and Szpirer, C. (1979) *J. Cell Sci.* 35, 267-279.
- Szpirer, J., Szpirer, C. and Wanson, J.-C. (1980) *Proc. Natl. Acad. Sci.* 77, 6616-6620.
- Tatarinov, Y.S. (1964) *Vop. Med. Khim.* 10, 584-589.
- Tchipyshova, T.A., Guelstein, V.I. and Bannikov, G.A. (1977) *Int. J. Cancer* 20, 388-393.

- Terranova, V.P., Rohrbach, D.H. and Martin, G.R. (1980) *Cell* 22, 719-726.
- Toftager-Larsen, K., Kjaersgaard, E., Jacobsen, J.C. and Nørgaard-Pedersen, B. (1980) *Clin. Chem.* 26, 1656-1659.
- Tumyan, B.G., Svet-Moldavsky, G.G. and Karmanova, N.V. (1975) *Nature* 255, 244-245.
- Tsukada, Y. and Hirai, H. (1975) *Ann. N.Y. Acad. Sci. USA* 259, 37-44.
- Tsukada, Y. and Hirai, H. (1976) In: *Onco-Developmental Gene Expression* (Fishman, W.H. and Sell, S. eds.) Academic Press, New York, pp. 639-646.
- Tsukada, Y., Richards, W.L., Becker, J.E., Potter, V.R. and Hirai, H. (1979) *Biochem. Biophys. Res. Commun.* 90, 439-446.
- Tsung, Y.-K., Milunsky, A. and Alpert, E. (1980) *J. Immunol. Meth.* 39, 363-368.
- Uotila, M., Engvall, E. and Ruoslahti, E. (1980) *Molec. Immunol.* 17, 791-794.
- Uotila, M., Ruoslahti, E. and Engvall, E. (1981) *J. Immunol. Meth.* 42, 11-15.
- Uriel, J., deNéchaud, B. and Dupiers, M. (1972) *Biochem. Biophys. Res. Commun.* 46, 1175-1180.
- Versée, V. and Bareil, A.O. (1979) *Biochem. J.* 179, 705-707.
- Versée, V., Zeeuws, R. and Bareil, A.O. (1981) *Arch. Int. Physiol. Biochem.* 89, B40-B41.
- Vlodavsky, I. and Gospodarowicz, D. (1981) *Nature* 289, 305-306.
- Watabe, H. (1971) *Cancer Res.* 31, 1192-1194.
- Watabe, H. (1974) *Int. J. Cancer* 13, 377-388.
- Watabe, H., Leffert, H.L. and Sell, S. (1976) In: *Onco-Developmental Gene Expression* (Fishman, W.H. and Sell, S. eds.) Academic Press, New York, pp. 123-130.
- Watanabe, A., Miyazaki, M. and Taketa, K. (1976) *Int. J. Cancer* 17, 518-524.
- Weller, E.M. (1976) In: *Onco-Developmental Gene Expression* (Fishman, W.H. and Sell, S. eds.) Academic Press, New York, pp. 655-661.
- Wepsic, H.T. and Sell, S. (1974) *Immunol. Cancer Progr. Exp. Tumor Res.* 19, 297-324.
- Yachnin, S. and Lester, E. (1976) *Clin. Exp. Immunol.* 26, 484-490.
- Yoo, T.J., Kuan, K. and Vestling, C.S. (1979) *Biochem. Biophys. Res. Commun.* 89, 491-496.

Zimmerman, E.F., Voorting-Hawking, M. and Michael, J.G. (1977) *Nature* 265, 354-356.

Zizkovsky, V. and Masopust, J. (1974) In: L'Alpha-Foetoproteine (Masseyeff, R. ed.) Colloque de L'INSERM, Nice, pp. 125-137.

